

A BIOASSAY SYSTEM FOR COLLECTING VOLATILES WHILE SIMULTANEOUSLY ATTRACTING TEPHRITID FRUIT FLIES

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(Received March 1, 1993; accepted May 25, 1993)

Abstract—A bioassay system was developed that permits the testing of various substrates for biological activity in a flight tunnel, while simultaneously collecting a portion of the volatiles from the attractive source for subsequent chemical identification and quantification. Bioassays of the response of virgin female Caribbean fruit flies, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), to volatiles released by calling males were conducted in a greenhouse under natural light cycles and fluctuating environmental conditions, similar to those in the field. Using this system, the periodicity of response of the female flies between 1300 and 1845 hr (EST) was tested. Fifty to 75% response occurred between 1700 and 1845 hr. Male pheromone release was greatest between 1500 and 1800 hr. Videotaped records of insects, taken between 1700 and 1800 hr as flies approached and entered the traps, were analyzed to interpret the communicative role of the volatiles released. Significantly more flies landed on and entered the pheromone-emitting trap than the control trap. There was no difference in the amount of time spent on the trap face, an indication that volatiles were attractants. The system described should be of general utility in determination of the attraction of pest fruit flies to suspected attractants.

Key Words—Bioassay system, flight tunnel, fruit flies, Caribbean fruit fly, *Anastrepha suspensa*, Diptera, Tephritidae, pheromone.

INTRODUCTION

The development of more effective lures for monitoring populations of pest fruit flies may be enhanced if test systems permit determination of the chemical(s)

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being released while simultaneously monitoring the relative attractive response of the insect to the chemical(s). Detailed knowledge of the environmental conditions that occur during the bioassay period may provide significant information relative to variation in fruit fly response to the chemical attractant. As part of continuing efforts to discover and improve fruit fly attractants, we designed a bioassay system that permits the testing of various substrates for biological activity in a flight tunnel, the collection of a portion of the volatiles from an attractive source for subsequent chemical identification, the automated recording of environmental conditions during the investigation, and the use of a video system to monitor fly activity on and in the trap. Our initial studies employed the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), to test the effectiveness of the bioassay system in an evaluation of response of the female fruit fly over time to pheromone released by conspecific males. Bioassays were conducted in an environment of purified air containing a minimal amount of volatile organics and under a light, temperature, and humidity regime similar to that available in the field.

METHODS AND MATERIALS

Chemical Attraction, Killing, and Evaluation Bioassay System. The bioassay system consists of five main subcomponents designed for specific tasks and allows certain parameters to be varied between experiments (Figure 1). The components include the bioassay flight tunnel (BFT), airflow system (AFS), air delivery system (ADS), insect isolation trap (IIT), and the volatile collection system (VCS). The bioassay system was contained in a greenhouse at the U.S.D.A. facility in Gainesville, FL.

Bioassay Flight Tunnel (BFT). The BFT was designed to operate similar to those used for insect pheromone research (Baker and Linn, 1984, and references therein). Briefly, the BFT is constructed out of 0.64-cm-thick clear acrylic Plexiglas and fused together using methylene chloride as a solvent making transparent and nonintruding joints (Figure 1A). The main flight tunnel body is a rectangular box $30.2 \times 30.2 \times 122$ cm with solid fixed sides and bottom, having flanged front and back ends. Two removable top panels, each with a small sliding 10×10 -cm door centered 15 cm from the end of each panel, allows for hand access into the tunnel. These two top panels (each 30.5 cm wide \times 61 cm long) are removable for cleaning the tunnel interior between experiments. The sliding doors on the top panels allow quick access into the tunnel during a bioassay for insertion or removal of insects and/or equipment with minimal disruption and contamination of the interior environment. Temperature within the BFT and greenhouse is controlled by two independent heating and cooling subsystems. For increases in temperature, an in-house closed-

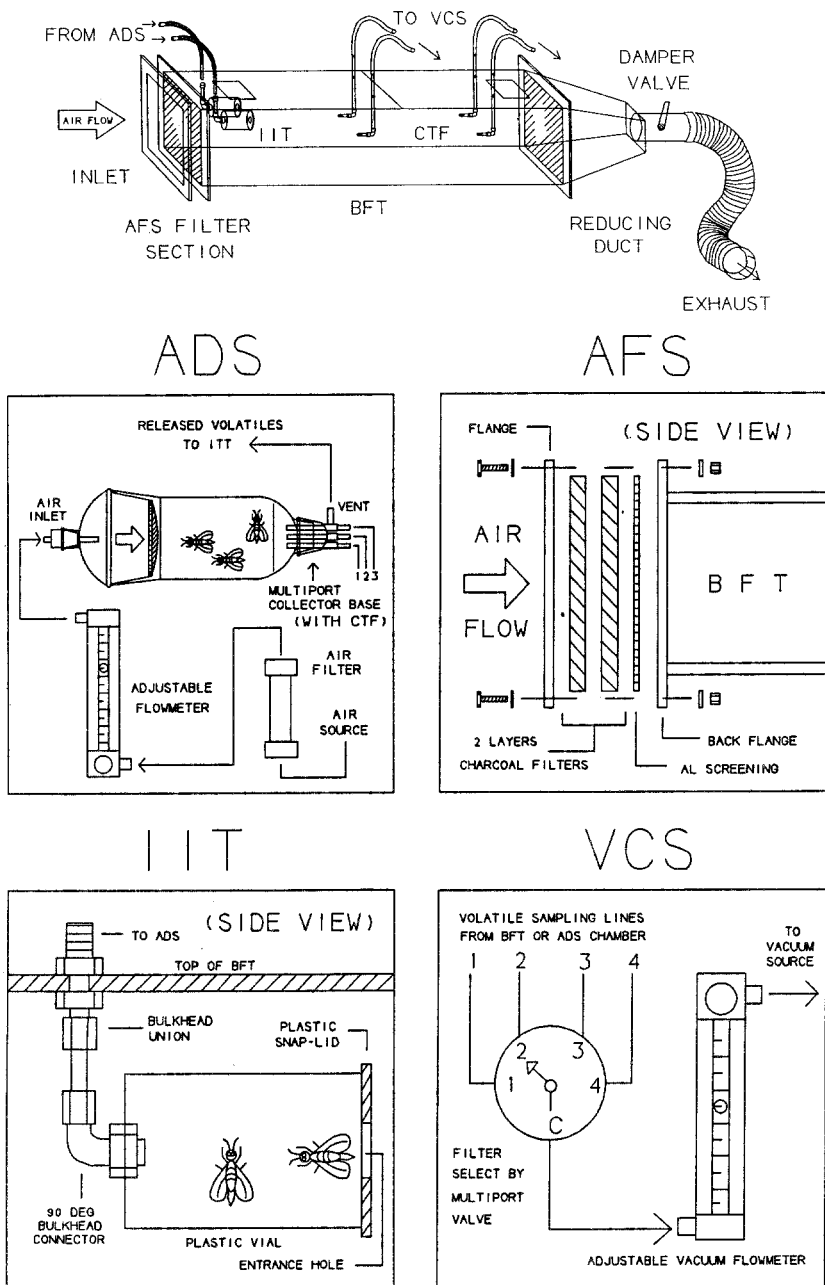


FIG. 1. The wind-tunnel bioassay system, which is comprised of (A) a bioassay flight tunnel (BFT); and (B) an air delivery system (ADS), an airflow system (AFS), two insect isolation traps (IIT), and a volatile collection system (VCS) with lines to collector trap filters (CTF) used to sample volatiles within the bioassay flight tunnel.

loop hot water circulation system with passive radiators is utilized. The lower limit for a desired temperature range is controlled by a heating-only thermostat and is used to maintain the temperature during the colder days of the year. The cooling system incorporates the use of evaporative water coolers and exhaust fans to lower temperatures to a desired setpoint. The upper limit of a temperature range is set by the cooling thermostat and, when that threshold is reached, two large exhaust blowers pull air out of the greenhouse. This causes fresh air to be drawn through evaporative coolers on the opposite wall through which water is constantly recirculated. During this process, water is vaporized into the incoming air which helps maintain a high relative humidity within the greenhouse.

Airflow System (AFS). The AFS consists of an air purification and diffusing system located at the upwind side and an air exhaust system located at the downwind side of the BFT (Figure 1B, AFS insert). The air purification method used is a modification of the charcoal-infused media system reported previously (Heath and Manukian, 1992). For the purpose of this study, two 1.27-cm-thick layers of 12.0-oz nonwoven fabric media infused with 150% activated carbon (18 oz of carbon per yard, P/N#ACF-NWPE-12-150P, Lewcote Corp., Millbury, Mississippi) were used. Each filter layer was cut to match the tunnel cross section and was secured to the intake side of the wind tunnel with a 30.5×30.5 -cm piece of standard aluminum window screening (1.5-mm square mesh). The material provides a means for producing a uniform laminar airflow across the tunnel cross section, in addition to purifying the incoming air.

The air exhaust system is comprised of an exhaust blower assembly and a reducing duct attached to the exhaust side or back-end of the wind tunnel. This is separated by another 30.5×30.5 -cm piece of the aluminum screening to confine insects to the tunnel, an air damper or baffle-type valve, a section of flexible corrugated plastic ducting, and finally an exhaust blower. The reducing duct is fabricated out of 0.64-cm-thick Plexiglas and serves to uniformly decrease the cross section of the tunnel down to an 8.89-cm-OD cylindrical port to which standard flexible plastic dryer-tube ducting is attached. This flexible ducting is used to connect the tunnel to the inlet of a high-capacity, 120-VAC, 1725 rpm, 28-cm-diameter radial wheel ("squirrel-cage") type exhaust blower motor capable of moving 40,500 liters/min of air (Grainger model 2C864, Chicago, Illinois). The blower is located several meters away from the tunnel to minimize any noise and vibrational effects on the tunnel generated from the blower motor. The air speed through the tunnel is controlled by increasing or decreasing the internal diameter of the outlet port at the end of the reducing duct by adjusting a damper inside this port. The wind speed is set by measuring the average velocity in the tunnel using a hot-wire type thermoanemometer (Kurz model 1440M-4, Davis Instruments Inc., Baltimore, Maryland) and positioning the damper until the desired speed is set. Experiments were conducted using a wind speed of 0.110 m/sec. Air pressure in the tunnel was measured using an elec-

tromechanical baratron tube differential pressure transducer (MKS Instruments model 223BD, Burlington, Mississippi) to determine if negative pressures were developed by exhausting more air by the blower than could pass through the carbon filter media.

Air Delivery System (ADS). The ADS is used to deliver a constant volume of air over the test substrate (e.g., calling males) and to entrain the volatiles for delivery to the bioassay wind tunnel without contamination (Figure 1B, ADS insert). A 45.5-liter portable air compressor capable of supplying 142 liters/min of air at 8.50 kg/cm² was used in this investigation. The maximum air supply needed for the ADS is a flow of 5.0 liters/min at a pressure of no more than 0.70 kg/cm² to prevent the separation or rupture of glass and soft Teflon tubing used in connecting all the components. A pressure regulator was used to reduce the source outlet pressure down to this level. The air coming from the compressor is then pushed through two in-line charcoal filters to remove organics from the air supply. The filters were made of 1.6-cm-OD (0.05-mm wall thickness) \times 12.7-cm-long stainless steel tubing and contain 28-mesh activated charcoal (Alfa Products, Danvers, Massachusetts) packed between two 325-mesh stainless steel screens held in place by 1.6- to 0.64-cm Swagelok reducing unions. Connected in line with these filters are two adjustable single-tube flowmeters hooked in parallel (Aalborg P/N# FM112-02C, Monsey, New York), which are used to set the flow rate of purified air that is delivered into the chamber containing the flies or the control chamber.

The 5-cm-OD \times 30-cm-long glass chamber with glass frit used to contain male flies has been previously described (Heath et al., 1992). Purified air is introduced upwind of the frit, which provides laminar airflow over the insects in the chamber. A multiport collector base located at the downwind end of the chamber contains a vent port for introduction of pheromone into the flight tunnel and three collector trap filters (see below) for pheromone collection. Airflow to the chamber containing male flies was maintained at 1 liter/min of which 250 ml/min of air was pulled through the collector trap filter and the remaining 750 ml/min was directed into the wind tunnel. Airflow to the chamber without flies was 750 ml/min. Connections from the flowmeters to the insect-holding chamber and from the vent port to the wind tunnel were made using a 40-cm piece of 0.64-cm-OD convoluted flex Teflon tubing (Berghof/America P/N# 14610-1/4", Concord, California) and standard 0.64-cm Swagelok fittings.

Insect Isolation Trap (IIT). Two insect isolation traps (Figure 1B, IIT insert) were symmetrically positioned 5.1 cm left and right of the centerline on the top panel of the wind tunnel 5.1 cm from the front (upstream) edge. The traps were cylindrical in shape and made from clear plastic 140-ml vials, 4.8 cm ID \times 8.6 cm long, with removable plastic snap lids (BioQuip, Gardena, California). Both were mounted horizontally, centered 10.2 cm from the top of the wind tunnel and 10.2 cm from the front end. A 1.1-cm-diameter hole was

drilled in the center of the bottom of each vial and attached to a 0.64-cm-ID Teflon 90-degree bulkhead union. This union was then attached to another 0.64-cm-ID bulkhead union mounted on the top panel (point at which the volatiles from the ADS enter the BFT, see above) using a 4.0-cm piece of 0.64-cm-OD stainless steel tubing as a support. A second 1.1-cm-diameter hole was drilled in the center of each removable lid, and then placed back onto each vial, pointing downstream. Lids were orange-colored to provide a visual cue (Greany et al., 1977). Interior surfaces of the insect isolation traps were coated with approximately 0.10 ml of a sugar-pesticide solution consisting of 10 g sucrose + 200 mg methomyl (98% AI, Reference #52543-98-1, DuPont Corp., Wilmington, Delaware) in 10 ml H₂O.

Volatile Collection System (VCS). The final component of the bioassay system is the VCS (Figure 1B, VCS insert), which is used for collecting a portion of released pheromone volatiles from flies inside the insect-holding chamber, as well as the downstream collection of volatiles at various locations inside the flight tunnel. Pheromone volatiles were collected on trap filters that were prepared by packing ca. 25 mg of Super-Q (Alltech Assoc. Inc., Deerfield, Illinois) in 4-cm-long \times 4.0-mm-ID glass tubes, resulting in a bed length of 5 mm. Two stainless steel frits were used to contain the adsorbent. The Super-Q filters were cleaned by Soxhlet extraction with methylene chloride for 24 hr prior to use. Volatiles collected on the filters were eluted with 100 μ l of methylene chloride, and then 1 μ g of *n*-tetradecane was added as internal standard for subsequent analyses.

Besides the three filters placed in the multiport collector base, additional filters were placed in the flight tunnel. These filters were attached to 0.64-cm Swagelok elbow fittings that were supported from the top of the bioassay wind tunnel at various locations by rigid 0.64-cm-OD Teflon tubing. This tubing was inserted through 0.64-cm holes cut in the top panel of the bioassay tunnel along its centerline at the front, middle, and back ends. The insertion length of the tubing can be adjusted vertically in the wind tunnel. Attached to the outside end of this tubing were various lengths of No. 15 Masterflex Tygon tubing (ca. 0.6 cm ID), which connected the filters to a VCS vacuum manifold assembly placed in the vicinity of the tunnel. This manifold assembly consisted of multiport switching valves, flowmeters, and a vacuum pump. This assembly enabled the selection of a filter to use for volatile collection and controlled the sampling rate for the collection. The selection of the individual collection filters located in the insect-holding chamber and at various locations in the BFT was done using four-way, multiport valves (Whitney #B-43ZF2, Swagelok Co., Solon, Ohio). Tygon tubing was used to make the connection from the filters to the vacuum source. The common outlet of the valve was attached to a vacuum source through a single tube adjustable flowmeter (Aalborg P/N# FM112-02C, Monsey, New

York) that was used to set the collection flow rate of the selected filter. Volatile collections made within the BFT were made with an airflow of 1 liter/min.

Analyses of Pheromone. Gas chromatographic analyses were conducted using a Hewlett-Packard model 5890A Series II gas chromatograph, equipped with a cool on-column capillary injector (septum injector) and flame ionization detector. Helium was used as the carrier gas at a linear flow of 18 cm/sec, and the column temperature was initially isothermal at 60°C for 2 min, then temperature programmed at 20°/min to 180°C. The chromatographic data was collected and processed using the Perkin-Elmer Nelson Turbochrom III software running on an IBM-type 386-PC system under MS-Windows 3.0. Capillary gas chromatography (CGC) was done using a retention gap column prior to the capillary column. This system permitted the injection of samples without concentration in 5–100 μ l of solvent (Grob, 1982; Murphy, 1989). A combination of three fused silica columns connected in series using GlasSeal connectors (Supelco Inc., Bellefonte, Pennsylvania) was used. The primary deactivated fused silica column, 8.0 cm long \times 0.5 mm ID, was connected between the injector and the retention gap column. This primary column permitted the use of 0.4-mm-OD stainless steel needles with a septum injector for on-column injections. The retention gap columns used were 10-m \times 0.25-mm-ID deactivated fused silica (Quadrex, New Haven, Connecticut). The analytical column used for analysis was a 30-m \times 0.25-mm-ID SE-30 purchased from Alltech Assoc. Inc. (Deerfield, Illinois). Confirmation of compound identity was obtained by mass spectroscopy using the SE-30 capillary column, operated as described above, coupled to a Finnigan Ion Trap mass spectrometer in either electron impact (EI-ITDMS) or chemical ionization (CI-ITDMS) mode. The reagent gas used for CI was isobutane.

Environmental and Experimental Monitoring. Environmental parameters were recorded using an Omega Engineering Inc. dual digital display thermohygrometer with linearized analog outputs for monitoring temperature and relative humidity (Omega model RH-411, Stamford, Connecticut), and a three-decade digital light meter (also with linearized analog outputs and RS-232C interface) covering a range from 0 to 50,000 lux utilizing a selenium photovoltaic detector with a 300-nm bandwidth centered at 570 nm for measuring light intensity during the experiments (Davis Instruments Inc., C/N# EH1191025, Baltimore, Maryland). The analog outputs of these instruments were connected to a Computerboards Inc. CIO-AD08 eight-channel analog to digital (A/D) data acquisition board (Mansfield, Massachusetts) inside a USDA assembled rack-mounted industrial Intel-based 80386 computer system used for data logging. The remote sensors used to measure temperature, humidity, and light intensity were connected to the A/D board through a Computerboards Inc. ISO-RACK08 signal conditioning board containing Analog Devices 5B-type analog isolation and signal conditioning modules (Norwood, Massachusetts) used to filter out

any noise from the sensors and amplify weak signals. The sampling rate of this system was 16,000 Hz (samples/sec) divided by the number of input channels used, in this case three, allowing for the collection of environmental data at 5300 samples a second per parameter. Data were averaged continuously and stored on the computer's hard disk once every 6 min, yielding 10 data points per hour for temperature, relative humidity, and light intensity.

Activity of the test insects on the trap was recorded using live remote NTSC video equipment. A Yashica model KX-90U 8-mm tape camcorder with 8 \times zoom, telephoto, macro, and autofocus lens (Kyocera Corp.) was mounted on a Panasonic model WV-7330 remote-controlled motorized three-axis camera pointing system (Panasonic Corp., Secaucus, New Jersey) placed next to the bioassay tunnel. This allowed for accurate pointing of the video camera at any object inside the tunnel from a remote location without disturbing insects inside the tunnel. All functions of the camera were also controlled remotely using a Sony Remote Commander model RM-95 LCD display remote control unit (Sony Corp.). The video signal of the camera was put through a Panasonic model WJ-810 time/date generator, which superimposed the video signal with accurate date and time information as well as an elapsed time counter. This combined signal was then recorded on VHS format T-160 tape on a Panasonic model PV-6000 portable VCR.

Bioassay Procedure. Caribbean fruit flies were obtained as pupae from the Florida Department of Agriculture, Division of Plant Industry in Gainesville, Florida. Adult flies were sorted by sex two days after eclosion and placed in screen cages (30 \times 30 \times 30 cm). Flies were maintained in the laboratory with a 12:12 hr light-dark photoperiod at room temperature and ambient relative humidity and were provided with water and food (3:1 refined cane sugar-hydrolyzed brewer's yeast). Flies were placed in the greenhouse one day prior to testing.

Bioassays were conducted using virgin sexually mature females and males (10–16-days old) during four time periods in the afternoon. An experiment consisted of placing 10 males in the insect-holding chamber at 1200 hr and venting any volatiles produced during this time period outdoors and away from the air inlet into the greenhouse and BFT. At 1300 hr, 20 virgin females were released from a vial in the BFT, while at the same time 75% of the volatiles from the 10 males were introduced into one of the insect isolation traps. Twenty-five percent of the volatiles released by males were collected on a collector trap filter for subsequent analyses of pheromonal components. At 1500 hr, females were removed from the BFT and IIT, and 20 more females were released. Pheromone collection was continued using a different collector trap filter. This procedure was repeated at 1700 hr and at 1800 hr. Bioassays were concluded at darkness (<2 lux), which typically occurred between 1830 and 1845 hr. A paired control IIT was included that had a flow of 750 ml/min of purified air

from a container similar to the insect-holding chamber, but containing only water and sugar in the same amounts as that provided to the males. This experiment was repeated six times, and each time the positions of the pheromone and control volatiles were switched to eliminate possible IIT location effects.

The relationship between male pheromone production and number of females responding was examined with correlation analysis using Proc Corr (SAS Institute, 1985). The number of females trapped per collection period was tested for correlation with amounts (nanograms per male per hour, adjusted for 1 liter/min total airflow) of each individual component and of total pheromone (i.e., sum of all individual components), and with the percent of each component produced per collection period. The behavior of the flies on the trap with pheromone volatiles versus the control trap was examined by viewing videotapes recorded during five of the trials. Data recorded included number of flies landing on a trap, amount of time females spent on the face of a trap, number of aggressive interactions between females on the face of a trap, and percent of landing flies that entered a trap. Data was analyzed by two-sample *t*-test using Proc TTest (SAS Institute, 1985).

RESULTS

System Performance. At a wind velocity of 0.25 m/sec, a volume of 1394 liters of purified air per minute (83,640 liters/hr) is passed through the tunnel. With this particular configuration, velocities up to 0.5 m/sec can be used to purify air through the charcoal filter media and maintain ambient pressures within the BFT. Measurements of pressure differences at a wind velocity of 0.5 m/sec were determined to be -1.21×10^{-5} kg/cm² (or -0.069 in. of H₂O) under ambient atmospheric pressure. Investigation of the airflow in the BFT using smoke demonstrated that a laminar airflow existed over the central portion of the BFT and a uniform air velocity was obtained throughout the length of the system. Plume geometry from the IITs using smoke indicated that mixing of the two plumes occurred at approximately 38 cm from the outlet holes when a flow of 750 ml/min was used through the IIT and a wind speed of 0.25 m/sec through the tunnel. Removal of organic contaminants using charcoal infused media provided a level of air purity similar to that previously reported (Heath and Manukian, 1992).

Pheromone Emission and Bioassay Results. Gas chromatographic analysis of volatiles collected from male flies contained previously identified compounds, i.e., ocimene, (Z)-3-nonenol, and (Z,Z)-3,6-nonadienol, suspensolide, (E,E)- α -farnesene, β -bisabolene, anastrephin, and epianastrephin (Chuman et al., 1988, Nation, 1990; Rocca et al., 1992). Chromatograms of collections made at the outlet of the insect-holding chamber were similar to that previously published

(Heath and Manukian, 1992). Analyses of the average amounts of total pheromone released and percentages of the pheromonal components released by males collected at the outlet of the insect-holding chamber are shown in Table 1. Based on analyses of volatiles collected inside the flight tunnel 30 cm downwind of the IIT, we found that approximately 4.4% of suspensolide, 2.3% of (*E,E*)- α -farnesene, 3.4% of β -bisabolene, 3.4% of anastrephin, and 2.5% of epianastrephin were present at this point in the BFT as compared with material collected at the outlet of the insect-holding chamber. The amounts of ocimene, (*Z*)-3-nonenol, and (*Z,Z*)-3,6-nonadienol collected could not be measured accurately using capillary gas chromatography; however, their presence was confirmed using mass spectroscopy. Total ion chromatograms of volatiles collected in the flight tunnel indicated that the purity of air using the charcoal-infused media was similar to that observed in our initial studies of the use of this material for air purification. No detectable pheromonal components were obtained in the analyses of collections made 105 cm downwind from the IIT.

Response of virgin females to the pheromone released by virgin male Caribbean fruit flies increased throughout the time periods tested. Based on the response of 120 female flies released during each of the four time periods, an average percent \pm standard error of females trapped per time period was: 34.2 ± 2.4 (1300–1500 hr); 61.7 ± 6.14 (1500–1700 hr); 60.0 ± 4.7 (1700–1800 hr) and 50.0 ± 2.9 (1800–1845 hr). Precise comparisons of these percentages are not possible since the lengths of the time periods are not equal. However, over 65% of the total response occurred between 1500 and 1800 hr. Of the 480 females tested, only two were trapped in the control IIT.

The number of female flies caught during each time period and the total amount of pheromone released by males for the six experiments conducted is shown in Figure 2. There were no significant correlations between the number of females trapped per hour and the amount of any of the individual components or the sum of all components produced per hour. There was a significant correlation, however, between the proportion of epianastrephin in the total pheromone blend produced and the number of females trapped per hour ($r = 0.428$, $df = 21$, $P = 0.05$), and that was the only component proportion to which the female response was significantly correlated. An examination of a scatterplot of percent epianastrephin and female response indicated that the relationship was most apparent during the first two collection periods (Figure 3).

Examination of environmental data recorded during the six experiments indicated a high degree of variability in light intensity, temperature, and humidity. This was largely due to the dynamic weather changes experienced daily in Florida during the time of year the experiments were conducted, which affect the conditions within the greenhouse. The occurrence of a severe storm and consequent early onset of darkness resulted in an earlier termination of both pheromone production and female response (see Figure 2, experiment 2). Light

TABLE 1. AVERAGE PERCENTAGES (\pm SD) OF PHEROMONAL COMPOUNDS AND AVERAGE AMOUNTS (\pm SD) OF TOTAL PHEROMONE BLEND RELEASED BY MALE FLIES DURING THE AFTERNOON^a

Time	Compound (%)						Total (ng/male/hr)
	Ocimene	Nonenols	Suspensolide	Farnesene	Bisabolene	Anastrephin	Epianastrephin
1300-1500	3.0 \pm 1.9	2.2 \pm 1.7	46.9 \pm 7.2	5.3 \pm 1.5	30.8 \pm 7.9	2.4 \pm 1.0	9.4 \pm 3.9
1500-1700	3.8 \pm 2.1	1.6 \pm 1.3	35.7 \pm 11.4	7.2 \pm 3.1	34.4 \pm 8.6	2.6 \pm 1.3	14.7 \pm 3.2
1700-1800	3.3 \pm 2.3	1.2 \pm 1.0	27.6 \pm 11.7	7.4 \pm 2.2	38.7 \pm 6.8	3.7 \pm 0.7	18.1 \pm 4.2
1800-1845	3.7 \pm 1.6	1.2 \pm 1.1	18.2 \pm 12.0	8.9 \pm 2.1	43.5 \pm 0.8	1.7 \pm 2.4	22.8 \pm 7.9
							144.4 \pm 22.0

^aCollections were made at the outlet of the insect holding chamber.

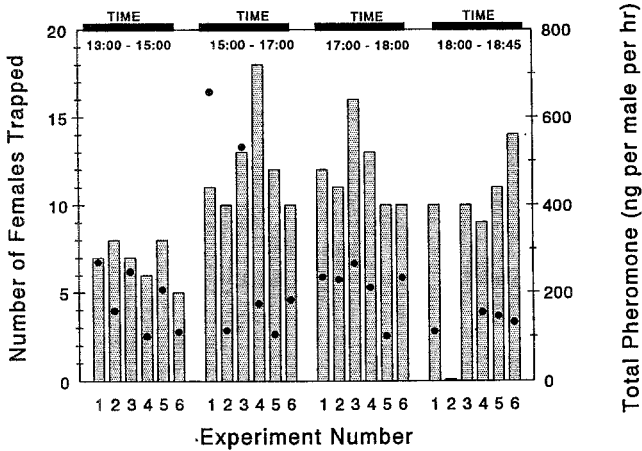


FIG. 2. The number of female *A. suspensa* trapped (bars) and the total amount of pheromone blend released by male *A. suspensa* (symbols) during six bioassays with simultaneous volatile collections. There were 20 females released during each time period.

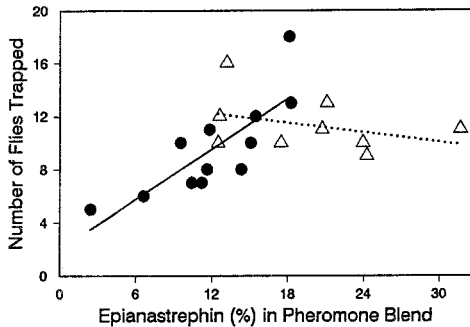


FIG. 3. Scatterplot of the number of female *A. suspensa* trapped in response to, and the percent epianastrephin in, pheromone released by male *A. suspensa*. The solid line represents tests run between 1300 and 1700 hr (two 2-hr tests), and the dotted line represents tests run between 1700 and 1845 hr (a 1 hr and a 45 min test).

intensity recorded during the 1700- to 1845-hr period for each experiment is shown in Figure 4. A representative data set of continuous measurements of temperature, humidity, and light intensity obtained during an experiment is shown in Figure 5. Considerable fluctuation in light intensity occurs between 1300 and 1600 hr. These fluctuations reflect the varying cloud coverage that occurred during a relatively sunny day. Light intensity decreased rapidly after 1600 hr, with darkness occurring at approximately 1840 hr. Temperature tended

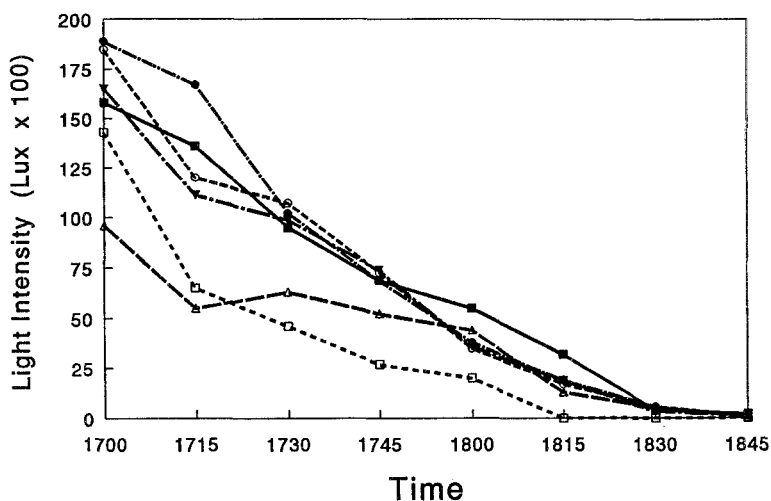


FIG. 4. Light intensities recorded at dusk during bioassays of response of female *A. suspensa* to volatiles from calling male *A. suspensa*.

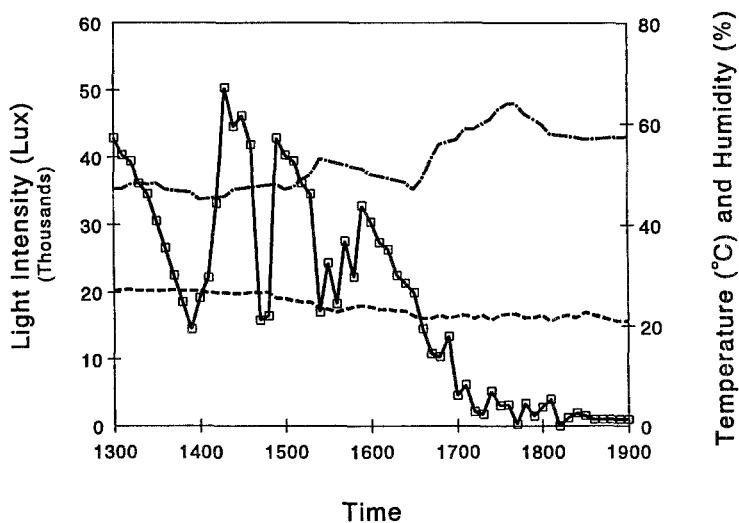


FIG. 5. An example of environmental data on temperature, humidity, and light intensity recorded during bioassays conducted in a greenhouse under simulated natural conditions.

to be fairly stable, with an overall drop in temperature throughout the test. Relative humidity tended to be inversely related to temperature, with an increase in relative humidity occurring as light intensity dropped after 1600 hr.

A review of the video taken from 1700 to 1800 hr, a time period of high female response in all trials, showed that there was a total of 109 landings on the traps. Significantly more landings occurred on pheromone-emitting traps ($18.2 \pm 3.8/\text{hr}$) than on control traps ($3.6 \pm 1.1/\text{hr}$; $t = 3.73$, $df = 4.7$, $P = 0.02$). There was no difference in the amount of time spent on the trap surface, with 41.3 ± 8.5 sec spent on the face of the pheromone-emitting trap versus 47.3 ± 18.2 sec on the control trap ($t = 0.3$, $df = 25$, $P = 0.77$). Females on the trap surface sometimes engaged in apparent aggressive interactions. Thirty-two percent of the landings resulted in aggressive encounters, and females sometimes left the trap surface after these interactions. Females that engaged in aggressive interactions were more likely to leave the trap than those without such encounters (48% vs. 37%, respectively). Of those flies that eventually went into the trap, those that engaged in fights spent significantly more time on the trap surface (90.1 ± 16.7 sec, $N = 15$) than those that went in without aggressive interactions (21.2 ± 4.3 sec, $N = 40$; $t = 4.0$, $df = 16$, $P = 0.001$). Sixty percent of the 91 landings on a pheromone-emitting trap ended with the fly entering the trap.

DISCUSSION

The long calling period of the male Caribbean fruit flies and the complexity of the volatile blend released by male flies during that time (Nation, 1990) have impeded the determination of the appropriate pheromone blend that could be used as an effective lure for female fruit flies in the field. Some individual components and blends have been tested in either laboratory bioassays or field trials (Nation, 1975, 1991), but only low levels of attraction have been recorded in those studies. The response of females flies was surprisingly high in the bioassay system, with 50–75% response occurring within all but the earliest testing period. The rapidity of the response enabled an examination of its periodicity and a simultaneous determination of the pheromone blend produced during the periods of greatest attraction. Environmental factors influence male pheromone production (Epsky and Heath, 1993), and these may also reduce or enhance female responsiveness regardless of pheromone blend produced.

The video records of insects as they approach and enter the traps can be used to interpret the communicative role of the chemical being tested. Simple recording of the number of insects trapped over time does not distinguish effects due to arrestants from effects due to attractants (Landolt et al., 1992): Suppose

two chemicals are being tested, neither one of which is an attractant capable of eliciting directed flight over a distance. Equal numbers of flies would land near both ports due to random movement. As flies wander about, they would be equally likely to enter either port. However, if one chemical is an arrestant, i.e., keeps insects in its vicinity, then flies in the control port are more likely to leave the area than those held by the arrestant. In this case, a chemical with no ability to attract insects over any distance could give the misleading appearance of being an attractant. Therefore, observations of behavior are essential in determining that a substance is an attractant. The significant difference in number of landings on the pheromone versus the control sites demonstrates that the male volatiles are not simple arrestants, although the data alone do not reveal over what distances the chemical signal might be effective. More information might be gained from further modification of the video system. Records of anemotaxis at further distances from traps could give insight into the range of the signal.

Numerous bioassay systems have been developed to determine behavioral response of insects to attractive sources. The system described here affords the simultaneous identification of volatile chemicals released, determination of attractiveness of these blends, automated record of environmental conditions that occurred during the investigation, and assessment of fly activity on and in the trap. Unique to this research is the adaptation of infused charcoal media for air purification of large volumes of air and the detailed design and description of the numerous components used to conduct the experiments. Additionally, this paper is the first description of response and periodicity of response by female Caribbean fruit flies to measured amounts of pheromone released by conspecific males. The use of the system described should be of general utility in determination of the attraction of pest fruit flies to suspected attractants.

Acknowledgments—We acknowledge B. Dueben (USDA/ARS, IABBBRL, Gainesville, Florida) for technical assistance, and J. Coffelt (USDA/ARS, Gainesville, Florida), D. Robacker (USDA/ARS, Weslaco, Texas) and T. Baker (University of California, Riverside) for critically reviewing the manuscript.

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